

FURTHER OBSERVATIONS ON MAMMALIAN CROSS-INNERVATED SKELETAL MUSCLE

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The experiments of Buller, Eccles & Eccles (1960*b*) demonstrated that the speed of contraction of certain hind-limb muscles of the cat was in part determined by the motor-nerve innervation of those muscles. The experiments then performed consisted essentially of dividing the motor nerve supplying one fast-contracting skeletal muscle (typically the flexor digitorum longus) and one slow-contracting muscle (typically the soleus) and connecting the proximal nerve stump which formerly supplied the slow muscle to the distal stump of the nerve formerly supplying the fast muscle and vice versa. Following re-innervation of the muscles (by the inappropriate motoneurons) the speed of contraction of muscles formerly fast was slowed, and the contraction of muscles formerly slow was speeded up. These original observations were largely confined to studies of isometric twitch responses and, while measurements were made of the apparent fusion frequencies in isometric tetani of the cross-innervated muscles, no detailed studies of the form of the isometric myogram were reported. As a result of these studies it was not possible to state with certainty what part or parts of the contractile mechanism had been influenced by the altered motor innervation, but it appeared probable, both from the original study and subsequent observations (Buller & Lewis, 1963), that the decline of active state (Hill, 1949) had been changed by cross-innervation. If only the fall of active state was altered by cross-innervation then the rate of rise of tension during the initial period of an isometric tetanus would be expected to remain that of the original muscle, since the degree of active state would be maintained constant by the repetitive stimulation, and the form of the myogram would be determined by the force-velocity relation and the series elastic element of the muscle (Hill, 1938; Katz, 1939; Wilkie, 1949, but see Jewell & Wilkie, 1958). The present paper presents a study of the isometric tetani in cross-innervated muscles, using the techniques previously reported (Buller & Lewis, 1964*c*). A preliminary communication of some of these results has already been published (Buller & Lewis, 1964*b*).

METHODS

Preliminary operations

Young adult cats were anaesthetized with intraperitoneal injections of pentobarbitone sodium (Nembutal). Under full aseptic conditions an incision was made through the lateral skin of the calf and the underside of the lateral gastrocnemius muscle gently dissected away from the upper surface of the soleus muscle to expose the nerve to soleus. The nerve to the flexor hallucis longus (F.H.L.) was found and the muscle itself was then identified medially, one of two procedures being followed. Either the two nerves, N soleus and N F.H.L., were divided and cross-united (Buller *et al.* 1960*b*) or the two nerves were divided and both connected to either the soleus muscle or the F.H.L. muscle. In control operations the motor nerves to the soleus and F.H.L. were cut and then resutured.

All animals received penicillin by injection post-operatively.

Acute experiments

Animals were used at intervals after the initial aseptic operations ranging from 7 to 25 months. No obvious differences were noted in the effects of cross-innervation within this period. The techniques used for the study of the isometric twitch and tetanus responses have been detailed in two previous papers (Buller & Lewis, 1964*c, d*).

Great care was always taken to maintain the temperature within the range 36.5–37.5° C and always to adjust the twitch responses to optimum (Buller, Eccles & Eccles, 1960*a*; Buller & Lewis, 1963).

RESULTS

Figure 1 illustrates our essential confirmation of the earlier observations of Buller *et al.* (1960*b*). It will be seen that as a result of cross-innervation the twitch response of F.H.L. is appreciably slowed while the twitch response of soleus is speeded. However, it must be stressed that in our experience the conversion of fast to slow muscle is rarely complete and the conversion of slow to fast muscle never complete as judged by the time to peak of the twitch contraction. Even in animals in whom the preliminary operation has been performed over 2 years previously the cross-innervated soleus of the cat never attains the twitch speed of a normal F.H.L. muscle. In all the subsequent experiments to be described comparable alterations in twitch speed to those illustrated in Fig. 1 were always seen as a result of cross-innervation.

In the original observations of Buller *et al.* (1960*b*) control re-innervations of muscle were performed in which the cut motor nerves were allowed to grow back into their original muscles. Provided a sufficient length of time was allowed between the preliminary operation and the acute experiment, normal twitch speeds for the muscle concerned were observed. We have confirmed these observations and in addition noted that the maximum rate of tension rise during an isometric tetanus of normally re-innervated muscle also shows a normal value.

Typical traces of the isometric tension rise (upper beam) and tension differentials (lower beam) (cf. Buller & Lewis, 1964*c*) are shown in Fig. 2

for both normal and re-innervated muscle. The total tetanic tensions developed by the re-innervated muscles were typically about 70 % of that expected for a normal muscle of the same weight (Buller & Lewis, 1964*d*). It is of some interest that in chronically denervated muscle the maximum rate of tension rise retains its normal value in both fast and slow muscle in spite of the prolonged twitch times (Lewis, 1962 and unpublished observations).

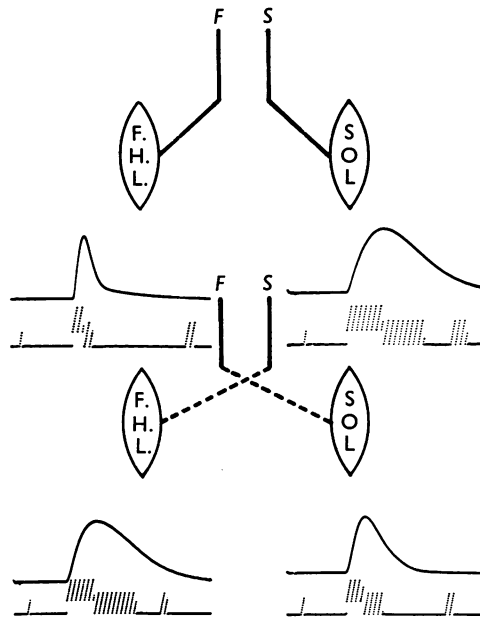


Fig. 1. Upper diagram and pair of records: isometric twitch responses from normal F.H.L. and soleus muscles of the cat. Lower diagram and pair of records: isometric twitches from cross-innervated F.H.L. and soleus muscle. *F* and *S* indicate the faster and slower conducting alpha motor axons respectively. The four groups of dots displayed under the isometric records in this and succeeding illustrations indicate the initial tension on the muscle, the time to peak tension (raised on a pedestal), the time to half relaxation and the twitch tension, respectively. For all four twitches each dot of the first group indicates 5 g of initial tension, each dot of the second and third groups indicate 1 msec, and each dot of the fourth group 5 g. It will be noted that the twitch of the cross-innervated F.H.L. muscle more closely resembles the normal soleus than the cross-innervated soleus twitch resembles the normal F.H.L.

The plot of time to peak twitch tension against percentage of maximum tetanic tension/msec (Buller & Lewis, 1964*c, d*) for normal and re-innervated muscles is illustrated in Fig. 3. Complete restitution of both normal values following re-innervation is apparent.

A further control was necessary for those experiments in which the double innervation of a single muscle was undertaken (see Methods). In

this type of experiment the stimulation of one of the two re-innervating motor nerves supplying a muscle resulted in the contraction of only those muscle fibres innervated by that motor nerve, the remaining muscle fibres being quiescent. The tension produced by the muscle when stimulated through either motor nerve singly was thus less than when both nerves were stimulated simultaneously; indeed the tension summation produced by stimulating both motor nerves simultaneously suggested that the muscle contained two independent groups of motor units, and that very few if any muscle fibres received an innervation from both motor nerves.

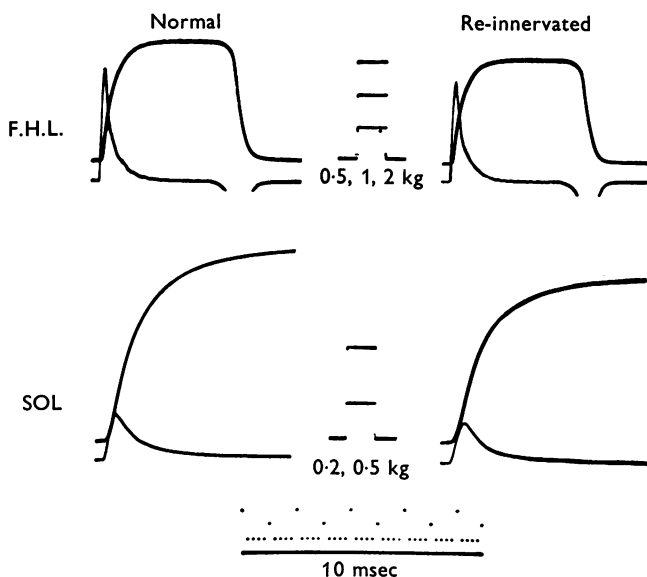


Fig. 2. Isometric tetani of normal and self-re-innervated F.H.L. and soleus muscles. In each pair of records the upper one indicates the isometric tension and the lower one the electrically differentiated tension record. The maximum height of the differentiated record is a measure of the maximum rate of tension development. Stimulation frequency for the two F.H.L. muscles was 500 imp/sec and for the two soleus muscles 250 imp/sec.

If only some of the motor units in a muscle contract, the common series elastic element, such as the tendon, will be stretched less than if all the muscle fibres are active and the contraction will be more nearly isometric. This greater isometricity will result in a smaller tetanus-twitch ratio and a more rapid time to peak tension in the twitch (cf. Brown & Matthews, 1960). It might also be expected to increase the maximum rate of tension development during an isometric tetanus, and this effect is illustrated in Fig. 4*a, b*. In these experiments ventral rootlet stimulation was compared with motor nerve stimulation, great care having been taken to denervate

the limb completely save for the two motor nerves used and to avoid stimulus spread from rootlet to rootlet. It was confirmed that the maximum rate of tension development increased as fewer and fewer motor units were activated. In Fig. 4*a* the smallest rootlet gave a value of 1.61 % P_0 /msec (% P_0 /msec is the percentage of the maximum tetanic tension developed per msec (Buller & Lewis, 1964*c*)) as compared with the motor nerve value of 1.36 % P_0 /msec for the maximum rate of tension development, while in Figure 4*b* the smallest rootlet produced a figure of 5.5 %

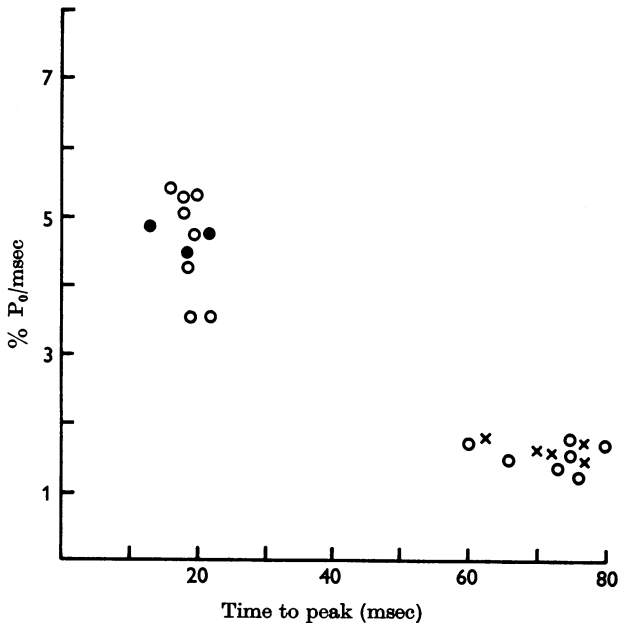


Fig. 3. Graph of the maximum rate of tension development (expressed as % P_0 /msec) during an isometric tetanus plotted against the twitch contraction time for normal and self-re-innervated muscles. The open circles indicate values for normal F.H.L. muscles (upper left) and normal soleus muscles (lower right). The filled circles indicate values for self-re-innervated F.H.L. muscles and the crosses values for self-re-innervated soleus muscles.

P_0 /msec as compared with a motor nerve value of 3.9 % P_0 /msec. Unfortunately the variability from experiment to experiment was considerable and the increase in the maximum rate of tension development during an isometric tetanus varied from 0 to 40 % for rootlet stimulation producing tetanic tensions of approximately 25 % maximum. We do not know the explanation of this variability, but it would seem reasonable to attribute at least some of it to the varying amount of common series elastic element included in the recording system.

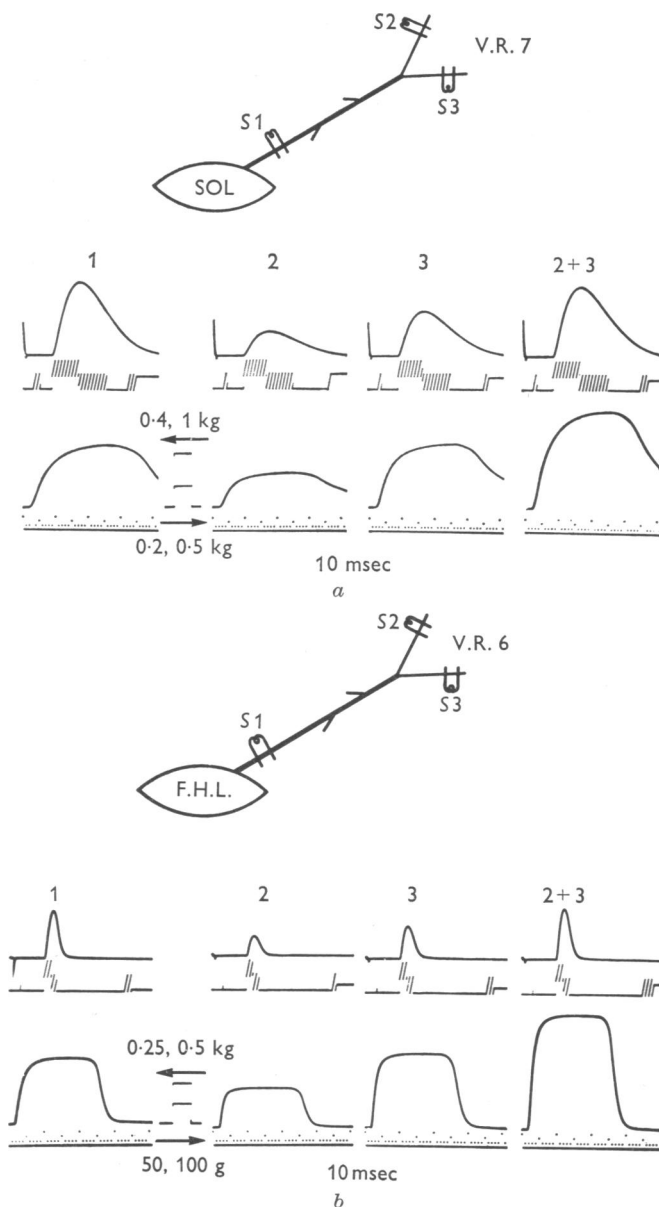


Fig. 4. To illustrate the effects of submaximal contractions of soleus muscle (4a) and F.H.L. muscle (4b). For both 4a and 4b S 1 indicates stimulating electrodes on the motor nerve through which maximal twitch and tetanic contractions are produced and illustrated under 1. S 2 and S 3 indicate stimulating electrodes on ventral rootlets by which submaximal contractions illustrated at 2 and 3 are produced. Stimulation of both rootlets simultaneously produces the mechanical responses indicated at 2+3. With smaller submaximal contractions the time to peak tension of the twitches (indicated by the second group of dots) diminishes and the maximal rate of tension rise during tetanic stimulation rises (see text).

Figures 5*a* and 5*b* show typical results from double innervation experiments of soleus muscle and F.H.L. muscle, respectively. In each case one innervation represents a normal re-innervation of the muscle by its own nerve while the other represents a cross-innervation. As stated above the two nerves appear to innervate separate populations of muscle fibres but the two groups of motor units have a common tendon and therefore share at least some of the muscle's series elastic element. The twitch responses via each of the motor nerves show the expected results. However, in soleus muscle (Fig. 5*a*) the maximum rates of tension development during optimal tetani are similar for both motor nerves, this being most apparent from the similar amplitude of the two differentiated tension records illustrated on the lower beams, which were photographed at the same gain. In the F.H.L. muscle (Fig. 5*b*), however, the maximum rates of tension rise are obviously different for the two groups of motor units, again shown most clearly in the differing amplitudes of the differentiated tension records.

Figure 6 shows a plot of the maximum rate of tension development during a tetanus against the time to peak of the twitch contractions for the cross-innervated and normal muscles. It may be seen that while the re-innervation of F.H.L. muscle fibres by soleus motoneurones produces a marked alteration in both the time to peak of twitch tension and the maximum rate of tension rise in an isometric tetanus, the re-innervation of soleus muscle fibres by F.H.L. motoneurones, while producing some speeding in time to peak of the twitch response, alters the maximum rate of tension rise very little.

It will be noted in Fig. 6 that the majority of the cross-innervated muscles (whether soleus or F.H.L.) gave values for the maximum rate of tension development during tetanic stimulation significantly higher (mean 2.08) than the values for normal soleus muscles (mean 1.62). However, as pointed out above, in calculating the maximum rate of tension rise allowance should be made for the lower tension exerted on the tendon by the cross-innervated parts of doubly innervated muscles as compared with the tensions developed by normal soleus muscles. Because of the variability referred to above no such correction has been attempted in Fig. 6 but undoubtedly at least some of the recorded values for cross-innervated muscles are artificially high.

A further effect of the cross-innervation of soleus muscle fibres by F.H.L. motoneurones first noted by Buller *et al.* (1960*b*) has been constantly observed, namely the increase in tetanus-twitch ratio. This is illustrated in Fig. 7*a*, which is from the same experiment as Fig. 5*a* but illustrates in addition the isometric twitch responses (and their differentials) photographed at the same gain as the tetanic contractions. It may be

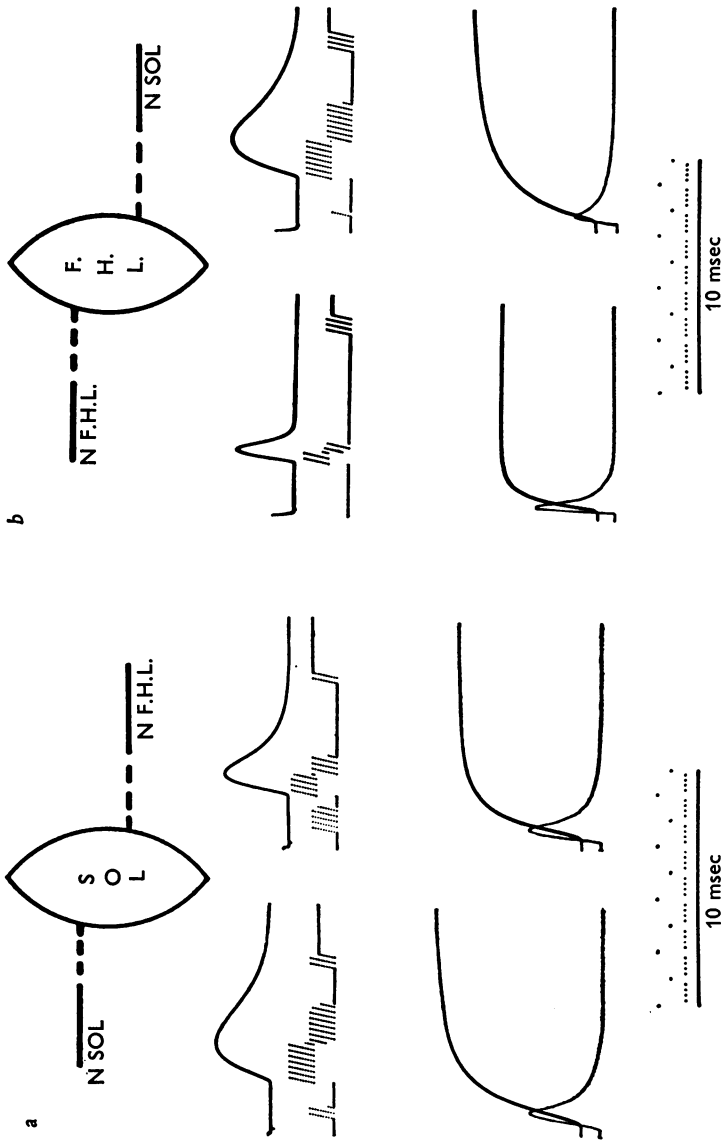


Fig. 5. Double innervation experiments of soleus muscle (5a) and flexor hallucis longus muscle (5b). In both a and b upper traces show the isometric twitch responses and the lower traces the isometric tetanic contractions and the differentiated tension records. In Fig. 5a the value of each dot in the initial tension group of the twitch obtained through the soleus nerve was 5 g and the value of each dot in the peak tension group also 5 g. For stimulation through the F.H.L. nerve each dot of initial tension represents 1 g and each dot of peak tension 2 g. In Fig. 5b for stimulation through both nerves each dot of initial tension represents 1 g and for peak tension each dot represents 4 g. Stimulation frequency for the tetani was 200 imp/sec.

seen that the tetanus-twitch ratio elicited via the former nerve to F.H.L. is more than double that found via the reconnected nerve to the soleus. In the present series the mean tetanus-twitch ratio for the soleus muscle re-innervated by F.H.L. motoneurones was 11.7 as compared with a mean of 5.6 for soleus motoneurones re-innervating the soleus muscle. A smaller difference also existed in the converse experiments where the mean value for the tetanus-twitch ratio of F.H.L. muscle innervated by soleus motoneurones was 4.3 while for F.H.L. muscle re-innervated by F.H.L. motoneurones the mean was 7.1. However, the considerable variation

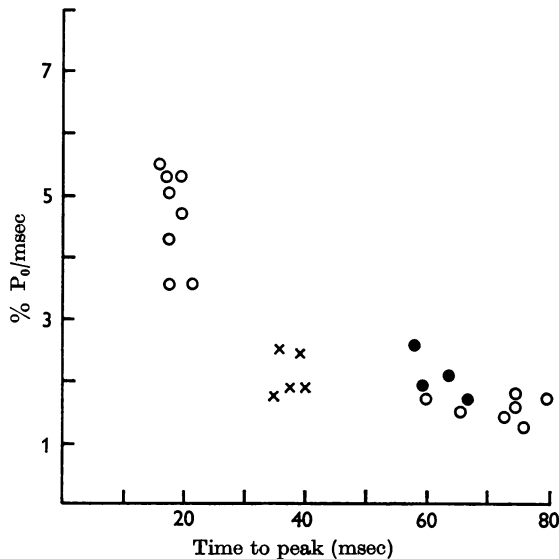


Fig. 6. Graph of the maximum rate of tension development (expressed as % P_0 /msec) during an isometric tetanus plotted against the twitch contraction time of normal and cross-re-innervated muscles. The open circles indicate values for normal F.H.L. muscles (upper left) and normal soleus muscles (lower right). The filled circles indicate values for F.H.L. muscles re-innervated by soleus motoneurones and the crosses values for soleus muscles re-innervated by F.H.L. motoneurones.

present in the tetanus twitch of normal muscles (Buller & Lewis, 1964*d*) precludes any significance being attached to the F.H.L. results with the present small number of observations.

Eccles, Eccles & Kozak (1962) suggested, without any experimental support, that the high tetanus ratios observed when F.H.L. motoneurones innervated soleus muscle might be due to the inability of some of the newly re-innervated end-plates to transmit single impulses. They supposed that some summation was necessary at such endings in order to generate a muscle action potential. We do not think this likely for three

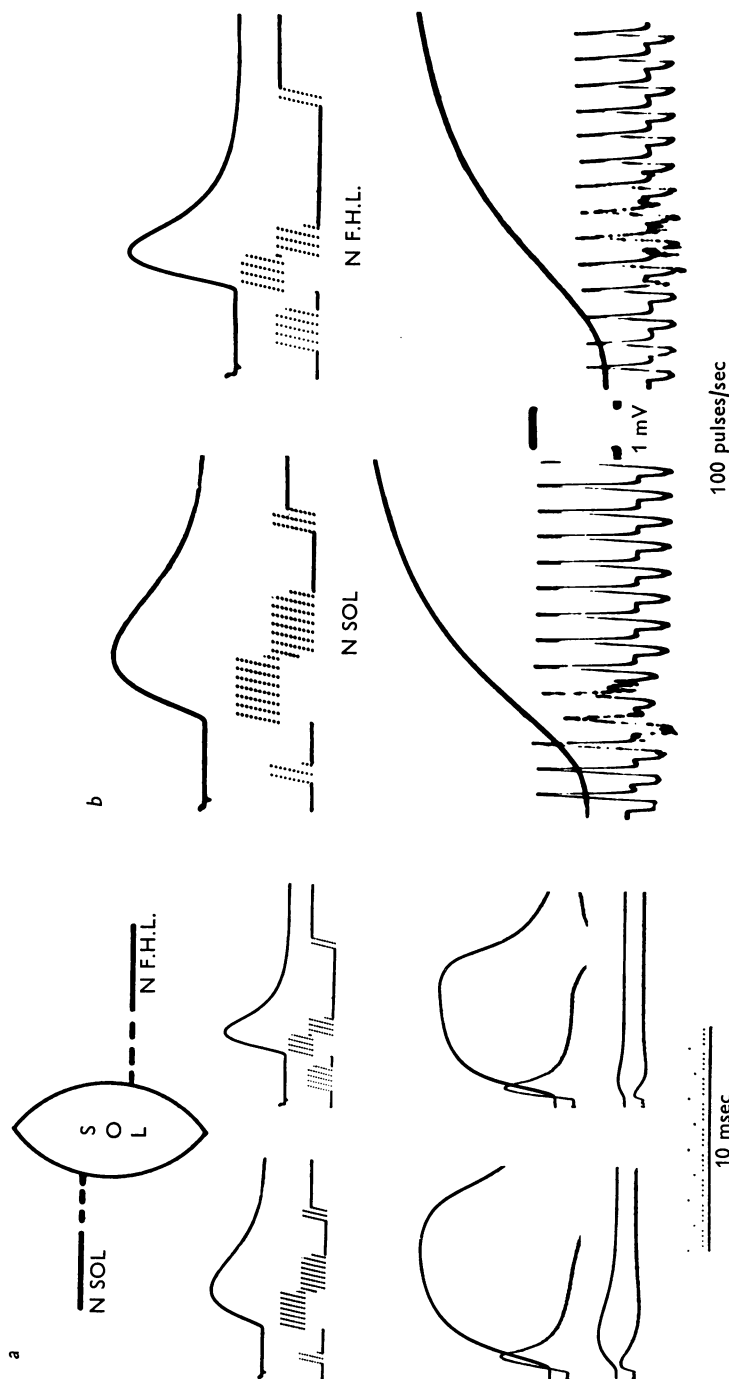


Fig. 7. *a*. The illustration is from the same muscle as Fig. 5*a* but below the tetanic records are illustrated the isometric twitch tension responses and their differentials photographed at the same gain as the tetanic responses. Note the considerably greater tetanus-twitch ratio elicited through the F.H.L. nerve as compared with the soleus nerve.

b. Above: Twitch responses as in Fig. 7*a*. Below: Upper trace; isometric records with stimulation at 100 imp/sec. Lower trace; action potentials recorded with a concentric needle electrode. The position of the needle in the muscle was moved between the stimulation of the nerve to soleus and stimulation of the F.H.L. nerve.

reasons. First, the high tetanus-twitch ratio does not decrease with time. Animals examined 2 years after cross-innervation operations still exhibit the phenomenon. Secondly, electrical recording of muscle action potentials following motor nerve stimulation shows little evidence of recruitment at frequencies of stimulation higher than those necessary to develop the peak tetanic tension. Figure 7*b* illustrates this point; a doubly innervated soleus muscle was stimulated repetitively at 100 pulses/sec first through one motor nerve and then through the other, while isometric tension (upper trace) and action potentials (lower trace) were recorded. No recruitment was seen. However, we have previously referred to the difficulties which may be experienced with needle electrodes owing to movement of the recording tip during contraction (Buller & Lewis, 1964*c*) and while we feel that this observation makes the suggestion of Eccles *et al.* (1962) unlikely we cannot completely exclude it.

Finally, in studying the effects of two stimuli applied to the motor nerves innervating normally or cross-re-innervated muscles it was observed that when the stimulus interval was shorter than the absolute refractory period of the muscle a small increase in the size of the twitch tension was frequently observed. This effect was comparable to that in kitten muscle (Buller & Lewis, 1964*d*, Fig. 3*a*) and was interpreted as being due to summation at the end-plates. In the present experiments typical twitch increments of 30 % were observed with such double stimuli but were seen not only in soleus muscles re-innervated by F.H.L. motoneurons but also in F.H.L. muscles re-innervated by either soleus or F.H.L. motoneurons. It would thus appear that even if allowance was made for the amount of facilitation that does occur the tetanus-twitch ratio of cross-innervated soleus muscle would remain abnormally high.

As the absolute refractory period for the system motor nerve fibre-end-plates-muscle fibres has been shown (Buller & Lewis, 1964*d*) to be different for normal soleus muscles (mean 1.6 msec) and normal F.H.L. muscles (mean 1.0 msec), comparable measurements were made on normally re-innervated and cross-re-innervated muscles. In both groups of re-innervated muscles the scatter of results was greater than for normal muscles. However, in normally re-innervated soleus muscle the mean absolute refractory period was 1.4 msec and in normal re-innervated F.H.L. muscles 0.9 msec. In cross-innervated soleus muscles the mean absolute refractory period was 1.1 msec and in cross-innervated F.H.L. muscles the mean value was also 1.1 msec.

DISCUSSION

The results above demonstrate that after cross-innervation mammalian fast muscle innervated by soleus motoneurones produces a slow twitch and a low maximum rate of tension development during an isometric tetanus (both characteristic of a normal slow muscle), while mammalian slow muscle innervated by F.H.L. motoneurones produces a faster twitch but retains a low maximum rate of tension development during an isometric tetanus. Thus, particularly in the case of slow muscle innervated by fast motoneurones there is evidence that the transitions brought about by cross-innervation may fall short of complete conversion of fast muscle to slow and vice versa. Indeed the present results indicate that at least one hybrid type of muscle is produced by such experiments, namely normally slow muscle re-innervated by phasic (fast) motoneurones. In such experiments not a single result has been reported in which cross-innervated slow muscle of the cat produced a twitch as fast, either in time to peak or half relaxation, as a normal or normally re-innervated fast muscle. The additional observation which has been made repeatedly on such cross-innervated slow muscles is that the tetanus-twitch ratio is high.

The results obtained on cross-innervated slow muscle might be explained using Hill's analytical model of muscle (Hill, 1949) by supposing that the new motor-nerve innervation induces a more rapid decay of active state in the muscle fibres following their excitation. Assuming that the force-velocity relation and the series elastic element of the muscle fibres remained unaltered, as is suggested by the unaltered maximum rate of tension development during an isometric tetanus, the tension developed during a twitch would be smaller (and hence the tetanus-twitch ratio larger) because of the more rapid decline of active state, and the time to peak tension and half decay of the twitch would be shortened for the same reason. A diagrammatic representation of this hypothesis was published earlier (Buller & Lewis, 1963).

The results obtained with cross-innervated fast muscle cannot be completely fitted by the same hypothesis. Slow motoneurones innervating F.H.L. muscle produce not only a slowed twitch but also a definitely reduced maximum rate of tension during an isometric tetanus. In short, the slow motoneurones have produced a more complete transition from normal fast towards normal slow muscle in the mechanical behaviour of the cross-innervated F.H.L. muscle, than fast motoneurones can produce in converting soleus muscle towards normal fast muscle. Again, using the Hill model these results suggest that as well as slowing the decline of active state (and thus lengthening the time to peak tension of the twitch) the new motor innervation has also altered either the force-velocity relation

or the series elastic element, or both, thereby slowing the maximum rate of tension development during an isometric tetanus. As already stated the tetanus-twitch ratios of such cross-innervated muscles, while tending to lie in the lower part of the normal range, were not distinctly different from normal.

The recent excellent paper by Close (1964) on the development of rat skeletal muscle is here pertinent. He has shown that during differentiation of mammalian fast skeletal muscle the force-velocity relation as well as the active state characteristics change, thereby confirming the earlier suggestion of Buller & Lewis (1964*a*). However, Close suggests that alterations in the time course of active state may be brought about either by changes in the force velocity properties independently of the force-velocity relation. While our present results could be conveniently interpreted in terms of this hypothesis, since the cross-innervated soleus muscle behaves as if the active state decay alone was altered, and the cross-innervated F.H.L. muscle behaves as if both the force-velocity relation and decay of active state were changed, nevertheless we feel that the nomenclature he uses is undesirable. As pointed out by Pringle (1960) the concept of active state must be rigidly defined to avoid ambiguity, and the subdivisions proposed by Close (1964) appear to militate against this. At the present time and in the absence of detailed knowledge of intracellular events it would appear wise to confine description to the experimental results rather than to elaborate on any one of the current models of muscle function.

The present observations can give no direct help in answering the question 'How do the motor nerves bring about their influence on muscle', though it is apparent that both the form of the twitch and the tetanic responses of the muscles may change as a result of cross-innervation. It would thus seem desirable to study both the twitch and tetanic responses of the muscles examined in any future investigations into how the motor nerve fibres bring about their effects on the contractile mechanism. This point is pertinent to the recent papers of both Eccles *et al.* (1962) and Vrbová (1963). These authors have attempted to investigate the influence of motor-nerve impulses on the contraction of mammalian skeletal muscle. The former group, using cats as the experimental animal, stimulated the popliteal nerve of one leg for various periods daily for durations of eight weeks and then compared the twitch characteristics of the activated muscles with the twitch characteristics of similar muscles on the control side. A slight slowing in the twitch times of the activated muscles was reported. However, no study was made of tetanic responses or tetanus-twitch ratio. Since the twitch speed may be altered in a number of ways, e.g. by changes in active state, force-velocity or series elastic element, the

results obtained by Eccles *et al.* (1962) cannot contribute directly to the understanding of the contractile mechanism of normal muscle, since it is not known if the total performance of the muscles studied (i.e. their tetanic responses and tetanus-twitch ratios) resembled either normal fast or slow muscles.

Vrbová (1963) studied the effects of tenotomy on the contractile responses of muscles. The majority of the experiments were carried out on rabbits and the deduction drawn from the experiments was that a reduction in the number of impulses down the soleus motor nerve led to an increase in the speed of the twitch response of the soleus muscle. Vrbová argued from this observation that the speeding of soleus observed in cross-innervation experiments might be due solely to a decreased number of nerve impulses passing down the new motor nerve.

Again, however, no systematic study of the tetanic contractions of the tenotomized muscles was made, and apart from a shorter twitch contraction time there was no evidence that the tenotomized soleus muscles behaved similarly to cross-innervated soleus muscles. We have re-investigated the effects of tenotomy in the rabbit (Buller & Lewis, 1964*e*) and while confirming that some increase of twitch speed occurs (though considerably less than that reported by Vrbová) it has been possible to demonstrate that the responses of tenotomized soleus muscle differ in important respects from those due to cross-innervation.

As a result of the experiments described in this paper we suggest that all motor nerves determine within the muscle fibres they innervate some factor analogous to the rate of decline of Hill's active state, and it is possible that this control might be brought about by the pattern of motor-nerve impulses (Ritchie & Wilkie, 1955).

In addition it is necessary to assume that the motoneurones can also exert some influence on the force-velocity relation of the muscle fibres they innervate, though these effects cannot be completely changed by cross-innervation. Whether the apparent inability of F.H.L. motoneurones to speed the force-velocity relation of soleus muscle following cross-innervation is due to some characteristic of the soleus muscle or to some failure of the motoneurones remains to be elucidated.

Finally, one general point relative to the future problem of solving how the motor nerve manages to influence the contractile mechanism may be raised. At present, as suggested by Buller *et al.* (1960*b*) two possible mechanisms may be envisaged which might act alone or in combination. These are the pattern of nerve impulses passing down the motor nerve and a hypothetical trophic substance secreted by the motoneurone and passing to the muscle fibres. As pointed out above at least two attempts have been made (Eccles *et al.* 1962; Vrbová, 1963) to elucidate the importance

of the pattern of nerve impulse activity, but in neither case was the possibility envisaged that the degree of activity to which the motoneurons was subjected might influence the metabolic activity of the motoneurons and thereby alter its production of a (hypothetical) trophic substance. In order to obtain a clearer understanding of the interrelation of nerve and muscle it will be necessary to design experiments which will overcome this criticism.

SUMMARY

1. Cross-innervation experiments have been performed on the soleus and flexor hallucis longus muscles of the cat and the earlier results of Buller *et al.* (1960*b*) have been confirmed.

2. In addition studies have been made of the maximum rate of tension development during isometric tetanic contractions.

3. These results indicate that, while the force-velocity relation of the flexor hallucis longus muscle may be altered by innervation with soleus motoneurons, the re-innervation of soleus muscle by flexor hallucis longus motoneurons produces no or very small change in the force-velocity relation.

4. Attention is drawn to the importance of studying both the twitch and tetanic contractions of muscle when investigating the contractile mechanism.

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